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(54) **Nutritional Composition**

(57) A nutritional composition is disclosed which comprises synergistic functional carbohydrates for prevention or treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health. The carbohydrates have a synergistic activity on promoting bifidobacteria in the intestine. Also disclosed are a method of production of the composition; use of the composition

in the manufacture of a functional food or medicament for the prevention or treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health; and a method of treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health which comprises administering an effective amount of the composition.

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Description

- [0001]** The present invention relates to a nutritional composition which comprises synergistic functional carbohydrates for prevention or treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health; a method of production of the composition; use of the composition in the manufacture of a functional food or medicament for the prevention or treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health; and a method of treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health which comprises administering an effective amount of the composition.
- [0002]** Within the context of this specification the word "comprises" is taken to mean "includes, among other things". It is not intended to be construed as "consists of only".
- [0003]** It has been suggested that there are health benefits associated with growth of bifidobacteria populations in the gut. These benefits include increased defence against pathogenic bacteria, stimulation of the immune system, and health benefits relating to the production of short chain fatty acids (SCFAs), as well as less abdominal sensation. All of these influence gut flora balance and gut health.
- [0004]** It is well known that infection by pathogenic bacteria can be detrimental to health. Examples of these bacteria include *Clostridium perfringens*, *C. difficile*, *Salmonella* and other enteropathogens.
- [0005]** In the past, infection by these harmful bacteria has been allowed to proceed until it must be treated by antibiotics. The antibiotics have a good effect on harmful bacteria. However, they suffer from the problem that they also kill populations of intestinal bacteria that are not harmful and that aid digestion of food. These bacterial populations are often referred to as "friendly".
- [0006]** Therefore, a need exists for a composition that is capable of preventing or combating infection by pathogenic bacteria, increasing defence against pathogenic bacteria, stimulating the immune system, and/or increasing short chain fatty acid production, all of which lead to promotion of gut flora balance and health.
- [0007]** The present invention addresses the problems set out above.
- [0008]** Remarkably, it has now been found that specific functional carbohydrates are capable of having a synergistic effect on the growth of bifidobacteria populations *in vitro* and *in vivo* in the gut.
- [0009]** Consequently, in a first aspect the present invention provides a nutritional composition for prevention or treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health which comprises at least two synergistic functional carbohydrates wherein a first carbohydrate is selected from the group which consists of inulin, fructooligosaccharide (FOS), galacto-oligosaccharide, soybean-gum, and a second carbohydrate is selected from the group which consists of xylooligosaccharide (XOS), gum, starch. The carbohydrates may be obtained commercially or more simply by the use of a natural source (eg: chicory as source of inulin).
- [0010]** In a second aspect the invention provides a method of production of the composition which comprises blending the components in the required amounts.
- [0011]** In a third aspect the invention provides use of a composition according to an embodiment of the invention in the manufacture of a functional food or medicament for the prevention or treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health
- [0012]** In a forth aspect the invention provides a method of treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health which comprises administering an effective amount of a composition according to an embodiment of the invention.
- [0013]** Preferably, an embodiment of a composition according to the present invention comprises fructooligosaccharide and a carbohydrate selected from the group which consists of xylooligosaccharide and acacia gum. More preferably it comprises fructooligosaccharide and acacia gum.
- [0014]** Preferably, an embodiment of a composition according to the present invention comprises about 1g to about 20g of a first carbohydrate and about 0.1 to about 20g of a second carbohydrate. More preferably, it comprises about 1g to about 3g of a first carbohydrate and about 0.2g to about 3g of a second carbohydrate for infant. Most preferably, it comprises about 2g to about 5g of a first carbohydrate and about 2g to about 5g of a second carbohydrate for adult. Although it will be apparent that there are no specific limitations except for what can reasonably be consumed and the price. This amounts given correspond to a daily dose, which may be divided into several servings in one day.
- [0015]** Preferably, an embodiment of the composition is formulated for human consumption and/or administration. Preferably, an alternative embodiment is formulated for consumption by a companion animal.
- [0016]** An advantage of the present invention is that it provides a composition that can be provided in a functional food product and which therefore does not require special administration.
- [0017]** Another advantage of the present invention is that it, but it does not adversely affect non-harmful intestinal bacteria or kill friendly bacteria present in the intestine.
- [0018]** Yet another advantage of the present invention is that it provides a decrease the daily amount of carbohydrates required in the gut to obtain stimulation of intestinal bifidobacteria and for promotion of the associated health benefit. The advantages that this decrease provides include reduction of the side effects (abdominal disturbance) induced by

the intake of some fermentable carbohydrates, and in some cases a reduction in cost.

[0019] Yet another advantage of the present invention is that it provides a composition of carbohydrates having various lengths of carbohydrate chains. This provides the advantage of modulating fermentation throughout the colon as the composition passes through it.

5 [0020] Additional features and advantages of the present invention are described in, and will be apparent from, the description of the presently preferred embodiments which are set out below with reference to the drawings in which:

[0021] Figure 1 shows the effect of various carbohydrates or blends thereof on faecal bifidobacteria in man.

10 [0022] According to the invention it is possible to stimulate specifically the growth of intestinal bifidobacteria or other lactic acid bacteria by the use of specific nondigestible carbohydrates (fibre or fibre-like substances), in vivo and in vitro. In humans and animals the bifidobacteria population is not the same from one individual to another and often comprises several different species of bifidobacteria.

[0023] The bifidobacteria enzymes implicated in fermentation of embodiments of a composition according to the invention are not the identical and differ depending on the physical and chemical structures of the carbohydrates. In addition, the bifidobacteria are not similar with regard to their enzymatic capacity, and thus, the ability to ferment one or another fibre is not the same from one bacteria to another.

15 [0024] Thus, a composition according to the invention which comprises a mixture of two or more carbohydrates promotes the growth of more bifidobacteria species than a single carbohydrate. Surprisingly, a synergy exists between carbohydrates on the stimulation of bifidobacteria and their health benefits (examples of carbohydrates include: fructo-oligosaccharides, galacto-oligosaccharide, soybean-gum, gum, starch).

20 [0025] Additional features of compositions according to the invention are given below. Comparative data showing results in human studies of using Fructo-oligosaccharides plus Acacia Gum or plus Xylo-oligosaccharides are given in the Examples. The data demonstrate an higher increase of bifidobacteria with a synergistic mix of carbohydrates than with single carbohydrate. These examples are described below.

25 [0026] In an embodiment, a nutritional composition preferably comprises a source of protein. Dietary protein is preferred as a source of protein. The dietary protein may be any suitable dietary protein; for example animal protein (such as milk protein, meat protein or egg protein); vegetable protein (such as soy protein, wheat protein, rice protein, and pea protein); a mixture of free amino acids; or a combination thereof. Milk proteins such as casein, whey proteins and soy proteins are particularly preferred.

[0027] The composition may also comprise a source of carbohydrates and/or a source of fat.

30 [0028] If the nutritional formula includes a fat source, the fat source preferably provides about 5% to about 55% of the energy of the nutritional formula; for example about 20% to about 50% of the energy. Lipid making up the fat source may be any suitable fat or fat mixture. Vegetable fat is particularly suitable; for example soy oil, palm oil, coconut oil, safflower oil, sunflower oil, corn oil, canola oil, lecithins, and the like. Animal fat such as milk fat may also be added if desired.

35 [0029] An additional source of carbohydrate may be added to the nutritional composition. It preferably provides about 40% to about 80% of the energy of the nutritional composition. Any suitable carbohydrate may be used, for example sucrose, lactose, glucose, fructose, corn syrup solids, maltodextrin, or a mixture thereof.

[0030] Additional dietary fibre may also be added if desired. If added, it preferably comprises up to about 5% of the energy of the nutritional composition. The dietary fibre may be from any suitable origin, including for example soy, pea, 40 oat, pectin, guar gum, gum arabic, fructooligosaccharide or a mixture thereof.

[0031] Suitable vitamins and minerals may be included in the nutritional composition in an amount to meet the appropriate guidelines.

45 [0032] One or more food grade emulsifiers may be included in the nutritional composition if desired; for example diacetyl tartaric acid esters of mono- and di-glycerides, lecithin and mono- or di-glycerides or a mixture thereof. Similarly suitable salts and/or stabilisers may be included.

[0033] The nutritional composition is preferably enterally administrable; for example in the form of a powder, a liquid concentrate, or a ready-to-drink beverage. If it is desired to produce a powdered nutritional formula, the homogenised mixture is transferred to a suitable drying apparatus such as a spray drier or freeze drier and converted to powder.

50 [0034] Alternatively, a common food product may be enriched with an embodiment of the composition. For example, a fermented milk, a yoghurt, a fresh cheese, a renneted milk, a confectionery bar, breakfast cereal flakes or bars, a drink, milk powder, soy-based product, non-milk fermented product or a nutritional supplement for clinical nutrition, infant formulae or baby food. Then, the amount of the composition added is preferably at least about 0.01% by weight.

[0035] An embodiment of the composition may be included in an article of confectionery, for example a sweet or sweetened beverage.

55 [0036] The following examples are given by way of illustration only and in no way should be construed as limiting the subject matter of the present application. Percentages and parts are by weight unless otherwise indicated.

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Example 1: Nutritional Composition.

[0037] A parallel design, consisting of 3 groups of 29 volunteers each, has been used:

FOS: 6g daily of (Raftilose P95N) during 6 weeks.

XOS: 0.4g daily of Xylo-oligo P95 during 6 weeks

XOS FOS: 4g daily of FOS and 0.2g daily of XOS, during 6 weeks

[0038] Before the 6 weeks of treatment, a wash-out period of 3 weeks was observed. The participants were then followed-up during 5 additional weeks after the end of treatment. During the wash-out and follow-up periods, the volunteers received a placebo.

[0039] During the complete test period, subjects refrained from eating fermented yoghurts and products containing bifidus.

[0040] In general the test subjects showed an increase of bifidobacteria counts, particularly those who had a low initial value. The average and median increases were also calculated. A formal statistical analysis gave the following results:

| FOS [log ₁₀ cfu/g] | Start | +1 Week | Difference | p-value |
|---|--------------|--------------------|-------------------|----------------|
| <i>Mean (t-test)</i> | 8.41 | 9.02 | +0.61 | 0.019 |

| XOS [log ₁₀ cfu/g] | | | | |
|---|------|------|-------|-------|
| <i>Mean (t-test)</i> | 7.43 | 8.11 | +0.68 | 0.042 |

| XOS FOS [log ₁₀ cfu/g] | | | | |
|---|------|------|-------|--------|
| <i>Mean (t-test)</i> | 7.39 | 8.85 | +1.45 | <0.001 |

[0041] Differences of bifidobacteria counts between start and first week of treatment

[0042] The results shown in the table provide clear evidence that FOS and XOS FOS significantly increase the average bifidobacteria counts. This is also the case for XOS, but to a lesser extent. The increase obtained with the mix XOS FOS is synergistically higher.

Example 2 :

[0043] The trial was designed, according to the protocol, to compare three groups of 32 resp. 31 subjects in parallel:

FOS:

[0044] 200 ml of skimmed milk with Raftilose P95®, 6g per serving.

Fibergum:

[0045] 200 ml of skimmed milk with Fibergum AS IRX®, 6g per serving.

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FOS + Fibergum:

[0046] 200 ml of skimmed milk with Raftilose P95® (3g per serving) and Fibergum TX® (3g per serving).

[0047] Faeces samples were tested 7, 21, 28, 49 and 71 days after the start of the study. The intervention period is between day 21 and 49. The change from day 21 to day 28 is of particular interest.

[0048] According to the protocol, a $+1.35 \log_{10}$ cfu/g of faeces increase of Bifidobacteria after one week of treatment is expected for the FOS + Fibergum group.

[0049] The individual changes in the FOS + Fibergum group from day 21 to day 28 are summarised below:

| Min. | 1st Qu. | Mean | 3rd Qu. | Max. |
|-------|---------|-------|---------|------|
| -0.33 | 0.07 | 1.384 | 1.42 | 6.73 |

[0050] The differences were analysed in two ways, first from a quantitative point of view, then more from a qualitative point of view:

[0051] Use of a robust location estimator, for example the M-estimator using Turkey's *bisquare* function gave an "average" of $0.307 \log_{10}$ cfu/g of faeces, close to the median value. A 95%-confidence interval was calculated by bootstrapping 1000 times (this is why the M-estimator was preferred compared to the median in this case). This gave as limits 0.14 and 1.00, that is an interval which does not include the value 0, indicating that the increase was statistically significant.

[0052] The "responders" were determined: we chose as criteria an increase of at least $+0.5 \log_{10}$ cfu/g of faeces. This was observed for 13 out of 29 subjects, which represented 44.8% of the volunteers. A 95%-confidence interval for this proportion was from 27 to 64%. We could say that at least 27% of the subjects respond to the diet provided, and it could be as much as 64%.

[0053] Using both approaches, we obtained a significant result.

[0054] For the Fibergum group, we obtained the following differences:

| Min. | 1st Qu. | Mean | 3rd Qu. | Max. |
|-------|---------|--------|---------|------|
| -6.26 | 0 | 0.6678 | 0.875 | 6.6 |

[0055] Here, median and mean differences were closer, and a t-test was appropriate. The average increase was at the limit of statistical significance (p -value = 0.09, 95%-CI: [-0.11, 1.45]).

[0056] Finally, for the FOS group, the differences were distributed as follows:

| Min. | 1st Qu. | Mean | 3rd Qu. | Max. |
|-------|---------|--------|---------|------|
| -4.04 | 0.0625 | 0.2853 | 0.655 | 4.31 |

Example 3 :

[0057] 100 volunteers were assigned randomly to 4 diet groups, but stratified for their amount of native intestinal Bifidobacteria before the trial, sex, age and average portions of fiber in the daily diet. The 4 diet groups are described below:

- **Control**
Reference Product
- **FOS+GUM**
Raftilose® 3g and Fibergum 3.56g per serving
- **Starch**
Resistant Starch 10g per serving
- **Blend**
Raftilose® 3g + Fibergum 3.56g + Resistant Starch 10g per serving

[0058] The primary analysis was to outcome is the effect on the amount of Bifidobacteria in the faeces. Counts of other micro-organisms were also analysed. A further analysis was carried out to determine changes of abdominal sensation (flatulencies, quality and number of stools) as assessed by the volunteers, followed by analysis of several short chain fatty acids measured in the faeces.

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[0059] The 100 volunteers were allocated to 4 groups as follows:

- Control: 13 subjects (8 females, 5 males)
- FOS+GUM: 29 subjects (19 females, 10 males)
- Starch: 29 subjects (19 females, 10 males)
- Blend: 29 subjects (19 females, 10 males)

[0060] Their average amount of bifidobacteria, age and portions of fiber were similar. There was one subject which clearly was not compliant (Group "FOS+GUM"), and its data were omitted.

[0061] It was set out to demonstrate that after 4 weeks of treatment, 50% of the subjects show an increase of at least $+0.5 \log_{10}$ cfu/g of faeces of Bifidobacteria. The lower boundaries of a 95%-Confidence Interval (CI) for the estimated proportion should be above 25%.

[0062] For the duration "Day 20 to Day 48", the following results were obtained of \log_{10} cfu bifidobacteria/g faeces (p) in (n) number of people. The number out of (n) having at least $0.5 \log_{10}$ cfu/g is shown in the first column ($+0.5$).

[0063] Bifidobacteria: Day 48 minus day 20

| | $+0.5$ | n | p | Lower | Upper |
|---------|--------|-----|------|-------|-------|
| Control | 3 | 13 | 23.1 | 6.2 | 54.0 |
| FOS+GUM | 9 | 27 | 33.3 | 17.2 | 54.0 |
| Starch | 7 | 27 | 25.9 | 11.9 | 46.6 |
| Blend | 13 | 27 | 48.1 | 29.2 | 67.6 |

[0064] In the above table, we see that, in the *Control* group, there were 3 out of 13 subjects that have an increase of at least $0.5 \log_{10}$ cfu/g of faeces. This represents 23.1%, with a 95%-confidence interval (CI) ranging from 6.2% up to 54.0%. For the Blend, the proportion was close to 50%, and the lower boundary of the 95%-CI is above 25%. Clearly there was a significant effect after 4w of consuming the blend.

[0065] After 1 week, the following results were obtained:

[0066] Bifidobacteria: Day 27 minus day 20

| | $+0.5$ | n | p | Lower | Upper |
|---------|--------|-----|------|-------|-------|
| Control | 5 | 13 | 38.5 | 15.1 | 67.7 |
| FOS+GUM | 12 | 28 | 42.9 | 25.0 | 62.6 |
| Starch | 9 | 28 | 32.1 | 16.6 | 52.4 |
| Blend | 11 | 29 | 37.9 | 21.3 | 57.6 |

[0067] Here, the effect of FOS+GUM was significant, and the blend was at the limit of statistical significance.

[0068] Changes in the amount of bifidobacteria were checked after 1 and 4 weeks of treatment to assess whether they related to the average portions of fiber eaten per subject. No striking association was found.

[0069] Similar experiments were carried out to determine the amounts of lactobacilli, bacteroides, enterobacteria and clostridium per fringens. It was surprisingly found that the number of these bacteria did not change significantly with respect to the different diets. This demonstrated the surprising result that an embodiment of the invention has the effect of specifically enhancing bifidobacteria. This result positively affects digestion, combats infection by pathogenic bacteria, stimulates the immune system, increases short chain fatty acid production and leads to promotion of gut flora balance and health.

[0070] It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

Claims

1. A nutritional composition for prevention or treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health which comprises at least two synergistic functional carbohydrates wherein a first carbohydrate is selected from the group which consists of inulin, fructooligosaccharide (FOS), galacto-oligosaccharide and soy-

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bean-gum, and a second carbohydrate is selected from the group which consists of xylooligosaccharide (XOS), fibergum, acacia gum and starch.

- 5 2. A nutritional composition according to claim 1 which comprises chicory as a source of inulin.
3. A composition according to claim 1 or 2 which comprises fructooligosaccharide and a carbohydrate selected from the group which consists of xylooligosaccharide and acacia gum.
- 10 4. A composition according to claim 1 or 2 which comprises fructooligosaccharide and acacia gum.
5. A composition according to any preceding claim which comprises about 1g to about 20g of the first carbohydrate and about 0.1 to about 20g of the second carbohydrate.
- 15 6. A composition according to any preceding claim which is formulated for human or companion animal consumption and/or administration.
7. A composition according to any preceding claim which comprises a third carbohydrate which is resistant starch.
- 20 8. A method of production of a composition according to any preceding claim which comprises blending the components in the required amounts.
9. Use of a composition according to any one of claims 1 to 7 in the manufacture of a functional food or medicament for the prevention or treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health.
- 25 10. A method of treatment of infection by pathogenic bacteria and/or promoting gut flora balance and health which comprises administering an effective amount of a composition according to any one of claims 1 to 7.

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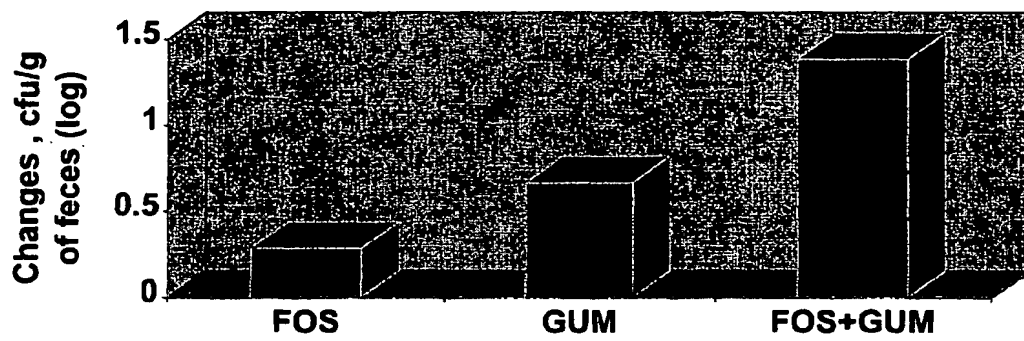
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Figure 1

Effect of various CHO or blend on fecal bifidobacteria in man



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PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

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| DOCUMENTS CONSIDERED TO BE RELEVANT | | | |
|---|---|----------------------------------|---|
| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.7) |
| X | US 5 444 054 A (GARLEB KEITH A ET AL) 22 August 1995 (1995-08-22) * tables 6,13,14 * * claims 1,7,10,13 * --- | 1,3-10 | A61K31/70 A61K31/715 A23L1/053 A23L1/0522 A23L1/308 A23L1/0528 |
| X | EP 0 756 828 A (NUTRICIA NV) 5 February 1997 (1997-02-05) * claims 1,4-6; example 1 * * column 3, line 31 - line 44 * --- | 1,6-10 | |
| X | US 5 721 004 A (JAMES SHERIANNE M) 24 February 1998 (1998-02-24) * claims 1,4,5,9 * * column 3, line 66 - column 4, line 23 * --- | 1,2,5,6, 8,10 | |
| X | EP 0 850 569 A (NESTLE SA) 1 July 1998 (1998-07-01) * claims 1,5-8 * * page 3, line 17 - line 25 * * page 5, line 4 - line 7 * * page 7, line 31 - line 33; example 5 * --- | 1,2,6, 8-10 | |
| | | | TECHNICAL FIELDS SEARCHED (Int.Cl.7) |
| | | | A23L A61K A23G |
| INCOMPLETE SEARCH | | | |
| <p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely : -</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search:</p> <p>see sheet C</p> | | | |
| Place of search | | Date of completion of the search | Examiner |
| THE HAGUE | | 10 January 2001 | Heezius, A |
| CATEGORY OF CITED DOCUMENTS | | | |
| <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>& : member of the same patent family, corresponding document</p> | | | |

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**INCOMPLETE SEARCH
SHEET C**

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Although claim 10 is directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Reason for the limitation of the search (non-patentable invention(s)):

Article 52 (4) EPC - Method for treatment of the human or animal body by therapy



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PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 00 11 5850

| DOCUMENTS CONSIDERED TO BE RELEVANT | | | CLASSIFICATION OF THE APPLICATION (Int.Cl.7) |
|-------------------------------------|--|-------------------|--|
| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | |
| A | GIBSON G R ET AL: "DIETARY MODULATION OF THE HUMAN COLONIC MICROBIOTA: INTRODUCING THE CONCEPT OF PREBIOTICS" JOURNAL OF NUTRITION, WISTAR INSTITUTE OF ANATOMY AND BIOLOGY, PHILADELPHIA, PA., US; vol. 125, no. 6, June 1995 (1995-06), pages 1401-1412, XP000972244 ISSN: 0022-3166 * page 1410, left-hand column * ----- | 1-10 | |
| | | | TECHNICAL FIELDS SEARCHED (Int.Cl.7) |
| | | | |

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**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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10-01-2001

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
|---|---|---------------------|----------------------------|---------------------|
| US 5444054 | A | 22-08-1995 | AU 689555 B | 02-04-1998 |
| | | | AU 1884495 A | 23-10-1995 |
| | | | CA 2187628 A | 12-10-1995 |
| | | | EP 0754001 A | 22-01-1997 |
| | | | NZ 281878 A | 24-02-1997 |
| | | | WO 9526646 A | 12-10-1995 |
| EP 0756828 | A | 05-02-1997 | AU 702989 B | 11-03-1999 |
| | | | AU 6087196 A | 06-02-1997 |
| | | | DE 69506095 D | 24-12-1998 |
| | | | DE 69506095 T | 24-06-1999 |
| | | | ES 2123903 T | 16-01-1999 |
| | | | US 5792754 A | 11-08-1998 |
| US 5721004 | A | 24-02-1998 | CA 2198681 A | 01-09-1997 |
| EP 0850569 | A | 01-07-1998 | AT 194461 T | 15-07-2000 |
| | | | AU 4853897 A | 25-06-1998 |
| | | | BR 9706448 A | 23-11-1999 |
| | | | CA 2221526 A | 24-06-1998 |
| | | | DE 69702491 D | 17-08-2000 |
| | | | ES 2148900 T | 16-10-2000 |
| | | | JP 10215805 A | 18-08-1998 |
| | | | NO 975915 A | 25-06-1998 |
| | | | NZ 329418 A | 29-07-1999 |
| | | | US 5952033 A | 14-09-1999 |
| | | | ZA 9711529 A | 22-06-1999 |

EPO FORM P459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82